

Sub C3 → --108. A primary or secondary cell having stably integrated into its genome:

a) exogenous DNA that encodes erythropoietin, and

b) DNA sequences that direct expression of the exogenous DNA in the primary or secondary cell.--

--109. The primary or secondary cell of claim 108, wherein said cell is selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

--110. The primary or secondary cell of claim 108, wherein said cell is of mammalian origin.--

31 --111. The primary or secondary cell of claim 110, wherein said cell is a human cell.--

--112. The primary or secondary cell of claim 108, further comprising DNA encoding a selectable marker.--

--113. The primary or secondary cell of claim 112, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.--

--114. The primary or secondary cell of claim 108, wherein said cell is selected from the group consisting of:

a) a primary or secondary cell that, prior to comprising said exogenous DNA, does not make or contain erythropoietin;

b) a primary or secondary cell that, prior to comprising said exogenous DNA, makes or contains erythropoietin in less than physiologically normal amounts or in defective form; and

c) a primary or secondary cell that, prior to comprising said exogenous DNA, makes or contains erythropoietin in physiologically normal amounts.--

--115. A primary or secondary cell comprising:

a) exogenous nucleic acid sequences that encode erythropoietin; and

b) nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in the primary or secondary cell,

wherein the nucleic acid sequences of (a) and (b) are present in the cell

episomally.--

--116. The primary or secondary cell of claim 115, wherein said cell is selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

--117. The primary or secondary cell of claim 115, wherein said cell is of mammalian origin.--

--118. The primary or secondary cell of claim 117, wherein said cell is a human cell.--

--119. The primary or secondary cell of claim 115, further comprising nucleic acid sequences encoding a selectable marker.--

--120. The primary or secondary cell of claim 119, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.--

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--121. The primary or secondary cell of claim 115, wherein said cell is selected from the group consisting of:

a) a primary or secondary cell that, prior to comprising said exogenous nucleic acid sequences, does not make or contain erythropoietin;

b) a primary or secondary cell that, prior to comprising said exogenous nucleic acid sequences, makes or contains erythropoietin in less than physiologically normal amounts or in defective form; and

c) a primary or secondary cell that, prior to comprising said exogenous nucleic acid sequences, makes or contains erythropoietin in physiologically normal amounts.--

--122. A clonal cell strain of secondary cells that express exogenous nucleic acid sequences encoding erythropoietin present therein.--

--123. The clonal cell strain of claim 122, wherein the exogenous nucleic acid sequences are stably incorporated into genomic DNA of the secondary cells.--

--124. The clonal cell strain of claim 122, wherein said secondary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

--125. The clonal cell strain of claim 122, wherein said secondary cells are of mammalian origin.--

--126. The clonal cell strain of claim 125, wherein said secondary cells are human cells.--

--127. The clonal cell strain of claim 122, wherein the exogenous nucleic acid sequences are present in the secondary cells episomally.--

--128. A heterogenous cell strain of secondary cells having stably incorporated into their genomes:

a) exogenous DNA encoding erythropoietin, and

b) DNA sequences that direct expression of the exogenous DNA in the secondary cells.--

--129. The heterogenous cell strain of claim 128, wherein the secondary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

--130. The heterogenous cell strain of claim 128, wherein said secondary cells are of mammalian origin.--

--131. The heterogenous cell strain of claim 130, wherein said secondary cells are human cells.--

--132. A mixture of cells consisting essentially of primary or secondary cells of claim 108 and primary or secondary cells that do not comprise said exogenous DNA.--

--133. A method of producing a clonal cell strain of secondary cells that express exogenous nucleic acid sequences encoding erythropoietin, said method comprising the steps of:

- B' Cont.
- a) providing a mixture of cells comprising primary cells;
  - b) introducing into primary cells provided in (a) a nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin and nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in the primary cells, thereby producing primary cells that express the exogenous nucleic acid sequences encoding erythropoietin; and
  - c) culturing a primary cell produced in (b) to produce a clonal cell strain of secondary cells that express the exogenous nucleic acid sequences encoding

erythropoietin.--

--134. The method of claim 133, wherein, in step (b), said nucleic acid molecule construct is introduced into primary cells provided in (a) by use of a viral vector.--

--135. The method of claim 134, wherein said viral vector is selected from the group consisting of retrovirus, herpes virus, adenovirus, adeno-associated virus, mumps virus, and polio virus vectors.--

--136. The method of claim 133, wherein said primary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

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Cont. --137. The method of claim 133, wherein said primary cells are of mammalian origin.--

--138. The method of claim 137, wherein said primary cells are human cells.--

Sub C4 --139. The method of claim 133, wherein, in step (b), nucleic acid sequences encoding a selectable marker are introduced into primary cells provided in (a).--

--140. The method of claim 139, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.--

Sub 5 → --141. The method of claim 133, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into primary cells provided in (a) by electroporation to produce at least one primary cell having the exogenous nucleic acid sequences stably integrated into genomic DNA.--

--142. The method of claim 141, wherein electroporation is carried out at an electroporation voltage of between 250 and 300 volts and a capacitance setting of approximately 960  $\mu$ Farads.--

B1 Cont. Sub 6 → --143. The method of claim 133, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into primary cells provided in (a) by microinjection.--



--144. The method of claim 133, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into primary cells provided in (a) by a transfection method selected from the group consisting of calcium phosphate precipitation, modified calcium phosphate precipitation, liposome fusion methodologies, receptor mediated transfer, micro-projectile bombardment, and polybrene precipitation.--

--145. The method of claim 133, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into genomic DNA by homologous recombination between DNA sequences present in the nucleic acid molecule construct and genomic DNA.--

*Spec 7* → --146. A method of producing a clonal cell strain of secondary cells that express exogenous nucleic acid sequences encoding erythropoietin, said method comprising the steps of:

- B1 Cont.*
- a) providing a mixture of cells comprising primary cells;
  - b) producing a population of secondary cells from primary cells provided in (a);
  - c) introducing into secondary cells produced in (b) a nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin and nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in

the secondary cells, thereby producing secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin; and

d) culturing a secondary cell produced in (c) to produce a clonal cell strain of secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin.--

--147. The method of claim 146, wherein, in step (c), said nucleic acid molecule construct is introduced into secondary cells produced in (b) by use of a viral vector.--

--148. The method of claim 147, wherein said viral vector is selected from the group consisting of retrovirus, herpes virus, adenovirus, adeno-associated virus, mumps virus, and polio virus vectors.--

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--149. The method of claim 146, wherein said primary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

--150. The method of claim 146, wherein said primary cells are of mammalian origin.--

--151. The method of claim 150, wherein said primary cells are human cells.--

*Sub C8* --152. The method of claim 146, wherein, in step (c), nucleic acid sequences encoding a selectable marker are introduced into secondary cells produced in (b).--

--153. The method of claim 152, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.--

*Sub C9* --154. The method of claim 146, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into secondary cells produced in (b) by electroporation to produce at least one secondary cell having the exogenous nucleic acid sequences stably integrated into genomic DNA.--

*B1 cont.* --155. The method of claim 154, wherein electroporation is carried out at an electroporation voltage of between 250 and 300 volts and a capacitance setting of approximately 960  $\mu$ Farads.--

Sub C10

--156. The method of claim 146, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into secondary cells produced in (b) by microinjection.--

--157. The method of claim 146, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into secondary cells produced in (b) by a transfection method selected from the group consisting of calcium phosphate precipitation, modified calcium phosphate precipitation, liposome fusion methodologies, receptor mediated transfer, micro-projectile bombardment, and polybrene precipitation.--

--158. The method of claim 146, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into genomic DNA by homologous recombination between DNA sequences present in the nucleic acid molecule construct and genomic DNA.--

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Sub C11

--159. A method of producing a heterogenous cell strain of secondary cells that express exogenous nucleic acid sequences encoding erythropoietin, said method comprising the steps of

a) providing a mixture of cells comprising primary cells;

b) introducing into primary cells provided in (a) a nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin and nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in the primary cells, thereby producing a mixture of primary cells that includes primary cells that express the exogenous nucleic acid sequences encoding erythropoietin;

c) culturing the product of (b) to produce a heterogenous cell strain of secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin.--

--160. The method of claim 159, wherein, in step (b), said nucleic acid molecule construct is introduced into primary cells provided in (a) by use of a viral vector.--

--161. The method of claim 160, wherein said viral vector is selected from the group consisting of retrovirus, herpes virus, adenovirus, adeno-associated virus, mumps virus, and polio virus vectors.--

--162. The method of claim 159, wherein said primary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

--163. The method of claim 159, wherein said primary cells are of mammalian origin.--

--164. The method of claim 163, wherein said primary cells are human cells.--

Sub C12 → --165. The method of claim 159, wherein, in step (b), nucleic acid sequences encoding a selectable marker are introduced into primary cells provided in (a).--

--166. The method of claim 165, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.--

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cont. Sub E13 → --167. The method of claim 159, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into primary cells provided in (a) by electroporation to produce at least one primary cell having the exogenous nucleic acid sequences stably integrated into genomic DNA.--

--168. The method of claim 167, wherein electroporation is carried out at an electroporation voltage of between 250 and 300 volts and a capacitance setting of approximately 960  $\mu$ Farads.--

Sub 14

--169. The method of claim 159, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into primary cells provided in (a) by microinjection.--

--170. The method of claim 159, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into primary cells provided in (a) by a transfection method selected from the group consisting of calcium phosphate precipitation, modified calcium phosphate precipitation, liposome fusion methodologies, receptor mediated transfer, micro-projectile bombardment, and polybrene precipitation.--

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--171. The method of claim 159, wherein, in step (b), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into genomic DNA by homologous recombination between nucleic acid sequences present in the nucleic acid molecule construct and genomic DNA.--

Subcls

--172. A method of producing a heterogenous cell strain of secondary cells that express exogenous nucleic acid sequences encoding erythropoietin, said method comprising the steps of:

- a) providing a mixture of cells comprising primary cells;
- b) producing a population of secondary cells from primary cells provided in (a);
- c) introducing into secondary cells produced in (b) a nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin and nucleic acid sequences that direct expression of the exogenous nucleic acid sequences in secondary cells, thereby producing a mixture of secondary cells that includes secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin; and
- d) culturing the product of (c) to produce a heterogenous cell strain of secondary cells that express the exogenous nucleic acid sequences encoding erythropoietin.--

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Cont.

--173. The method of claim 172, wherein, in step (c), said nucleic acid molecule construct is introduced into secondary cells produced in (b) by use of a viral vector.--

--174. The method of claim 173, wherein said viral vector is selected from the group consisting of retrovirus, herpes virus, adenovirus, adeno-associated virus, mumps virus, and polio virus vectors.--



--175. The method of claim 172, wherein said primary cells are selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.--

--176. The method of claim 172, wherein said primary cells are of mammalian origin.--

--177. The method of claim 172, wherein said primary cells are human cells.--

*Sub 176* > --178. The method of claim 172, wherein, in step (c), nucleic acid sequences encoding a selectable marker are introduced into secondary cells produced in (b).--

*B1 Cont.* --179. The method of claim 178, wherein said selectable marker is selected from the group consisting of one that confers nutritional auxotrophy, one that confers resistance to a cytotoxic agent, and one that results in expression of a surface protein.--

*Sub 175* > --180. The method of claim 172, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into secondary cells produced in (b) by electroporation to produce at least one secondary cell having the exogenous nucleic acid sequences stably integrated into

genomic DNA.--

--181. The method of claim 180, wherein electroporation is carried out at an electroporation voltage of between 250 and 300 volts and a capacitance setting of approximately 960  $\mu$ Farads.--

Sub C185

--182. The method of claim 172, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into secondary cells produced in (b) by microinjection.--

--183. The method of claim 172, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into secondary cells produced in (b) by a method selected from the group consisting of calcium phosphate precipitation, modified calcium phosphate precipitation, liposome fusion methodologies, receptor mediated transfer, micro-projectile bombardment, and polybrene precipitation.--

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Cont.

--184. The method of claim 172, wherein, in step (c), the nucleic acid molecule construct comprising exogenous nucleic acid sequences encoding erythropoietin is introduced into genomic DNA by homologous recombination between DNA sequences

present in the nucleic acid molecule construct and genomic DNA.--

--185. A method of producing a clonal cell strain of secondary fibroblasts of mammalian origin that express exogenous DNA encoding erythropoietin, said method comprising the steps of:

- a) providing primary fibroblasts of mammalian origin;
- b) producing a population of secondary fibroblasts from the primary fibroblasts provided in (a);
- c) combining the secondary fibroblasts of mammalian origin produced in (b) with a DNA construct comprising:
  - i) exogenous DNA encoding erythropoietin to be expressed in the fibroblasts; and
  - ii) DNA sequences of non-retroviral origin that direct expression of the exogenous DNA in the fibroblasts;
- d) subjecting the combination produced in (c) to electroporation to transfect the vector into the secondary fibroblasts of mammalian origin, thereby producing a mixture of transfected secondary fibroblasts of mammalian origin and nontransfected secondary fibroblasts of mammalian origin;
- e) isolating a transfected secondary fibroblast of mammalian origin produced in (d); and

f) culturing the transfected secondary fibroblast of mammalian origin isolated in (e) to produce of a clonal population of cells consisting essentially of transfected secondary fibroblasts of mammalian origin that express the exogenous DNA encoding erythropoietin.--

--186. A method of producing an antibody specific for erythropoietin, said method comprising introducing into a mammal a transfected primary or secondary cell of claim 108, and isolating polyclonal sera comprising antibodies specific for erythropoietin from said mammal.--

--187. An antibody produced by the method of claim 186.--

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Cont.  
--188. A method of producing a monoclonal antibody specific for erythropoietin, said method comprising introducing into a non-human mammal a transfected primary or secondary cell of claim 108, removing splenocytes from said non-human mammal, fusing said splenocytes with myeloma cells to produce hybridoma cells, and isolating a hybridoma cell that produces a monoclonal antibody specific for erythropoietin.--

--189. A monoclonal antibody produced by the method of claim 188.--